12-10-85

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metribuzin reregistration data package: a 9 week study to establish dose-time effect on the thyroid in rats. EPA ID #3125-270, EPA Record #156176, EPA Accession #257924, Caswell #33D, Shaugnessy #101101-4, Tox Branch Project #209.

TO: Robert J. Taylor, PM #25
Herbicide-Fungicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C) 12/10/85

THRU: Laurence D. Chitlik, DABT Quang Bui 12/11/85
Section Head, Review Section V
Toxicology Branch/HED (TS-769C)
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ACTION REQUESTED: Review of a 9 week feeding study to establish the dose-time-effect of Metribuzin on the thyroid in rats.

RECOMENDATION: This study is classified as Core Supplementary Data, since only males were examined and the histopathology was limited in scope; furthermore, these data are ancillary and, as such, do not meet any regulatory requirement.

The treatment of male rats with DIC 1468 revealed systemic effects in all four dose groups, however, effects other than body weight and food consumption were generally not dose related. The systemic No Observed Effect Level (NOEL) for this study is below 35 ppm, the lowest dose tested.

The main thrust of this study was to establish the dose-time-effect relationship for DIC 1468 in the thyroid. From the data presented the investigators assumed ". . . that peripheral deiodination of thyroxine to triiodothyronine is impaired after administration of up to 900 ppm DIC 1468, so that a secondary increase in thyroid functioning takes place." However, this assumption is difficult to follow from the evidence presented. The conclusion is that treatment with DIC 1468 appears to have an effect on thyroid function but the mechanism and biological significance remain uncertain.

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I. STUDY TYPE: Thyroid effects study - dietary administration

Study Identification: DIC 1468 (Metribuzin, Sencor®)
Subchronic Toxicological Study to Establish the Dose-Time-Effect Relationship for the Thyroid
(Nine-week feeding study)

EPA Identification Numbers: EPA ID #3125-270
EPA Record #156176
EPA Accession #257924
Caswell No. 330
Shaughnessy #101101-4
CAS 21087-64-9
CFR 180.332
Tox Project No. 209

Sponsor: Mobay Chemical Corporation

Testing Laboratory: Bayer AG
Institute of Toxicology
Wuppertal - Elberfeld

Study Numbers: Bayer Report No. 11231
Mobay Ag Chem. No. 82561

Study Date: October 19, 1982

Study Authors: Dr. F. Krotlinger
Dr. O. Vogel

Test Material: DIC 1468 (also known as Metribuzin, Sencor®)
Purity = 93.3%
Batch Eg. 1/80

Dosages: Control (0), 35, 100, 300, and 900 ppm mixed in Altromin® powdered feed
(feed manufactured by Altromin GmbH, Lage).

Test Animals: Male SPF rats, strain Wistar TNO W.74.
Bred by Winkelmann, Borchen.
At start of study: 6 to 7 weeks old
mean starting weight = 106 gm

II. MATERIALS AND METHODS: A copy of the Materials and Methods section from the investigators' report is appended:

This study was designed to assess the effects of DIC 1468 on male rats from a 9-week feeding, specifically to establish the dose-time-effect relationship for thyroid changes.

This study generally conforms to the 1982 Pesticide Assessment Guidelines, however, the following comments and highlights are noted:

According to the investigators: the active ingredient was checked by "analysis" at the start of the study, the stability of the active ingredient in the feed was determined and the active ingredient content in the food was rechecked. However, no data were provided in the study report to support these statements.

The treated animals received Altromin® powder feed (with test compound) and tap water ad libitum. The treatment was for 9 weeks, with an additional 3-week post-observation period.

Test groups consisted of 60 male rats each with an additional 20 rats added to the control, 300, and 900 ppm groups for radioimmunoassays (RIA) 7 and 21 days after the end of treatment.

Animals were checked daily, body weights recorded weekly, and weekly food consumption determined (by weighing uneaten food).

Clinical chemistry (of blood) was conducted on 10 rats per dose group on days 7, 21, and 63. Tests included: total thyroxine (2 methods), cholesterol, total protein, calcium, inorganic phosphate, and magnesium. Methodology was given by reference only.

Thyroid function tests were conducted on 10 animals per group on days 7, 21, and 63; also, 7 and 21 days after end of treatment, 10 animals per group from the extra animals in the control, 300, and 900 ppm groups were examined. Iodine (^{131}I) accumulation in the thyroid was established along with concentrations of triiodothyronine (T_3) and tetraiodothyronine (T_4) in the serum by RIA. The procedure is outlined in the Materials and Methods section appended (thyroids were weighed also).

Necropsies were conducted on the 10 animals used to collect blood for the clinical chemistry tests at days 7, 21, and 63. The thyroid, liver, and adrenals were weighed. Tissue samples were taken of liver, adrenals, thyroid, skeletal muscle, and any organ with a macroscopic alteration and histological examinations were conducted.

III. RESULTS

A. Clinical Observations

According to the investigators: "During the study period rats administered DIC 1468 in doses of up to 900 ppm in their food did not differ in appearance and behavior from the controls." No data were presented to substantiate this statement.

B. Food Consumption

The investigators did not provide individual data but provided mean total and mean weekly animal data. Table I below presents these data (all animals in study were included).

Table I. Food Consumption (gm/animal/day) During Treatment Period[a]

Dose (ppm):	Control	35	100	300	900
	16.7	16.4	17.6	16.6	15.7

[a] = Data extracted from Bayer AG Report No. 11231, Appendix.

The high-dose group animals consumed slightly less food than did the control during the treatment period.

C. Body Weight

The investigators provided mean, mean graphed, and individual animal data. The data for mean body weights are presented in Table 2 below (all animals in study were included).

Table II. Mean Body Weight (gms)[a]

	WEEK												
Dose (ppm)	0	1	2	3	4	5	6	7	8	9	10	11	12
Control	107	133	175	204	232	255	274	281	298	300	300	310	314
35	106	139**	174	200	229	254	271	283	296	304	-	-	-
100	106	135	174	200	228	253	268	279	294	300	-	-	-
300	105	127**	167**	193**	221**	244*	262*	273	289	291	297	300	303
900	105	119**	159**	184**	207**	229**	245**	260**	272**	278**	285	298	301

* = $p < 0.05$ as compared to control.
** = $p < 0.01$ as compared to control.
[a] = Data extracted from Bayer AG Report No. 11231, Table 2 and Appendix.

There was a dose-related decrease in body weight in the treated groups, as compared to controls, with statistically significant differences from control shown for the 300 ppm test group for weeks 1 through 6 and for the high dose group for weeks 1 through 9 (entire treatment period). Body weights for the 300 and 900 ppm groups were followed after treatment stopped and in both groups body weights became more equivalent to the controls than during the treatment period.

D. Mortality

No animals were reported to have died during the study period.

E. Clinical Laboratory Investigations

The investigators provided mean and individual animal data. Table III below presents the clincal chemistry results (tests were conducted on 10 animals per group, retro-orbital venous plexus blood samples).

Table III. Clinical Chemistry[a]

	Protein (gm/L)			Cholesterol (mmol/L)			Inorganic PO_4 (mmol/L)		
Day:	7	21	63	7	21	63	7	21	63
Dose (ppm)									
Control	54.9	57.5	61.9	1.68	1.80	1.86	2.26	2.71	2.07
35	54.8	57.4	62.3	1.68	1.94	1.73	2.71**	2.62*	2.19
100	56.7*	57.3	61.4	1.72	2.05*	1.84*	2.73**	2.64	2.07
300	56.5	57.7	61.6	1.91*	1.89	1.94	2.89**	2.73	2.11
900	57.0**	57.2	63.1	1.88	2.11**	1.95	2.62**	2.57	2.19
	T_4† (nmol/L)			Mg (mmol/L)			Ca (mmol/L)		
Control	86	80	64	0.82	0.88	0.75	3.12	2.99	2.75
35	126**	117**	99**	0.87	0.84	0.82*	3.03*	2.99	2.83*
100	121**	119**	92**	0.79	0.80*	0.69	3.10	3.05	2.74
300	130**	105**	86**	0.79	0.80**	0.62**	3.14	3.03	2.82
900	110**	82	95**	0.91	0.85	0.70*	3.19	2.99	2.85*

* $p < 0.05$ as compared to control
** $p < 0.01$ as compared to control
† = Total thyroxine

[a] = Data extracted from Bayer AG Report 11231, Tables 3, 4, 5 and Appendix.

The blood levels of protein were increased for the 100, 300 and 900 ppm test groups at day 7; however, significant increases were not seen on subsequent sampling days. Cholesterol levels were slightly increased over control, but none of the changes was of biological significance (although certain values were statistically significant). Inorganic phosphate levels were increased over control in all test groups on day 7; however, no biologically significant differences were seen on days 21 and 63. The total thyroxine (T_4) levels were statistically significantly increased over control in all dose groups on days 7, 21 and 63 (except for the high dose on day 21), however, no dose relationship was apparent. None of the noted differences from controls for magnesium and calcium levels of the treated groups was considered biologically meaningful although statistical significarce was noted for some.

F. Specific Thyroid Tests

1. Storage capacity of the thyroid

The investigators measured the storage capacity of the thyroid gland by the use of radiolabeled iodine (^{131}I). Table IV below presents the results of these measurements. The investigators provided mean and individual animal data for 10 animals per group (other than those animals used for clinical chemistry evaluations).

Table IV. Thyroid Tests[a]

	Organ Weight (mg)			Iodine Quantity (%)+			Iodine Conc. (%/mg)++		
Day:	7	21	63	7	21	63	7	21	63
Dose (ppm)									
Control	12.23	14.67	15.03	5.10	6.29	10.69	0.42	0.43	0.71
35	11.82	13.08	16.77*	6.01	7.40	17.57**	0.51	0.57**	1.04**
100	10.36*	13.42	16.60	7.06*	6.97	14.64**	0.67**	0.51*	0.88**
300	8.79**	13.81	19.09**	7.16**	6.50	16.19**	0.81**	0.47	0.85
900	8.69**	13.44	20.66**	6.67**	6.17	14.61**	0.77**	0.46	0.70

* = $p < 0.05$ as compared to control.
** = $p < 0.01$ as compared to control.
\+ = "the quantity of radioiodine in the thyroid as percentage of administered radioactivity."
++ = "the concentration of radioiodine in the thyroid as percentage of administered radioactivity per mg thyroid."
[a] = Data extracted from Bayer AG Report No. 11231, Tables 6, 7, 8, and Appendix.

Thyroid weights on day 7 were decreased in a dose related manner; on day 21 there were slight decreases in all test groups but no dose response relationship was evident; on day 63 there were increases at all levels, being dose related at 300 and 900 ppm. The quantity of radioiodine in the thyroid (as a percentage of administered radioactivity) increased over control in all dose groups on day 7, was not biologically significantly different on day 21, and increased over control in all 4 dose groups on day 63. When the iodine content was expressed on a concentration basis (%/mg), there were increases above control for all treated groups at all intervals (except 900 ppm on day 63) and for days 21 and 63, the increases were inversely related to dose, reflecting the increased organ weights of the 300 and 900 ppm groups compared to those of the 35 and 100 ppm groups.

Table V below shows the thyroid data for the control, 300 and 900 ppm test groups 7 and 21 days after end of treatment on 10 animals per group (of the added 20 animals per group).

Table V. Thyroid Tests - 7 and 21 Days Post-treatment[a]

	Organ Wts (mg)		Iodine Quant. (%)		Iodine Conc. (%/mg)	
Day:	7	21	7	21	7	21
Dose (ppm)						
Control	18.66	15.21	14.81	12.16	0.81	0.79
300	18.28	17.36	13.96	13.81	0.75	0.81
900	17.69	18.30**	15.59	15.01*	0.88	0.83

* = $p < 0.05$ as compared to control.
** = $p < 0.01$ as compared to control.
[a] = Data extracted from Bayer Report No. 11231, Tables 9, 10 and Appendix.

There are no biologically significant differences between control and the 300 and 900 ppm test groups in the 3 measurements 7 days from end of treatment. However, 21 days after end of treatment, there is a dose related increase in organ weight and in the quantity and concentration of radioiodine in the thyroid.

2. Radioimmunoassays (RIA)

The levels of triiodothyronine (T_3) and thyroxine (T_4) were determined by RIA, 7, 21, and 63 days after study initiation. Method used was stated as the Corning immo Phase® T_3 ^{125}iodine and T_4 ^{125}iodine radioimmunoassay test system, this was the only information provided (other than sample size). Table VI below presents the results of these measurements. The investigators provided mean and individual animal data for 10 animals per group (other than those animals used for clinical chemistry and storage capacity of the thyroid measurements).

Table VI: Radioimmunoassays[a]

	T_3 (ng/100mL)			T_4 (ug/100 ml)			T_3/T_4 ($\times 10^{-2}$)[†]		
Day:	7	21	63	7	21	63	7	21	63
Dose (ppm)									
Control	92.61	66.51	68.09	6.04	4.66	4.87	1.53	1.43	1.40
35	74.84**	61.11	64.02	7.35*	6.74**	7.30**	1.02	0.91	0.88
100	78.71*	74.75	65.84	7.86**	7.35**	7.77**	1.00	1.02	0.85
300	74.64**	72.97	64.36	8.12**	7.05**	7.56**	0.92	1.04	0.85
900	73.84**	92.21**	59.22*	6.14	4.92	6.06**	1.20	1.87	0.98

* = $p < 0.05$ as compared to control.
** = $p < 0.01$ as compared to control.
[†] = T_3/T_4 ratios were calculated by the reviewer, with both values in ug/100 ml.
[a] = Data extracted from Bayer AG Report No. 11231, Tables 11, 12, 13 and Appendix.

At day 7, the T_3 levels were decreased in all dose groups without any apparent relationship to dose, and T_4 levels increased in the 35, 100 and 300 ppm groups. At day 21, T_3 was increased at 100 ppm and above and T_4 was increased in all groups, except the highest dose. At day 63, there was a decrease in T_3 levels, significant for the 900 ppm group, and an increase in T_4 levels in all treated groups (all statistically significant). The variability in the control levels as seen above made it difficult to interpret the findings in the treated animals. The T_3/T_4 ratios were decreased for all dose groups at all times points (except for the high dose on day 21).

RIA's of T_3 and T_4 were conducted 7 and 21 days after end of treatment. Table VII below presents the results of these measurements of 10 animals per group (of the other 10 animals of the added 20 animals per group).

Table VII. Radioimmunoassays - Post Treatment[a]

	T_3 (ng/100 mL)		T_4 (ug/100 mL)		T_3/T_4 ($\times 10^{-2}$)[†]	
Day:	7	21	7	21	7	21
Dose (ppm)						
Control	74.23	54.56	4.95	3.80	1.50	1.44
300	66.11	56.45	6.08*	4.29	1.09	1.32
900	62.77*	71.89**	6.70**	4.96**	0.94	1.45

* = $p < 0.05$ as compared to control.
** = $p < 0.01$ as compared to control.
[†] = T_3/T_4 ratios were calculated by the reviewer, with both values in ug/100 ml.
[a] = Data extracted from Bayer AG Report No. 11231, Tables 14, 15 and Appendix.

At day 7, T_3 levels decreased and T_4 levels increased, while at day 21 both T_3 and T_4 levels increased. The T_3/T_4 ratios are decreased at both time points (except for the high dose at day 21). Variability of controls from day 7 to 21 is again noted.

G. Necropsy Observations

According to the investigators: "The autopsy of the rats sacrificed after 7, 21 and 63 study days did not reveal any indications of specific damage in the groups up to 900 ppm." (these were the animals used for clinical chemistry evaluations). No data were presented to substantiate this statement.

1. Organ Weights

The investigators measured the organ weights of the thyroid, liver, and adrenal glands. Tables VIII and IX present the results of these measurements (of the animals used for clinical chemistry evaluations).

Table VIII.. Absolute Organ Weights (mg)[a]

	Body Wt (gm)			Thyroid			Liver			Adrenals		
Day:	7	21	63	7	21	63	7	21	63	7	21	63
Dose (ppm)												
Control	136	211	315	13	13	16	5677	8287	8883	24	34	38
35	139	209	308	11*	14	18	5847	8527	9839	25	31	36
100	133	206	308	10	14	16	5461	8163	10219	25	34	35
300	132	195**	304	9**	13	18	5551	7852	9362	24	30*	36
900	120**	188**	286	9**	13	16	4963**	7934	9524	20*	31	37

* = $p < 0.05$ as compared to control.
** = $p < 0.01$ as compared to control.

[a] = Data extracted from Bayer AG Report No. 11231, Tables 16, 17, 18 and Appendix.

Table IX. Relative Organ Weights (mg/100 gm bw)[a]

	Thyroid			Liver			Adrenals		
Day:	7	21	63	7	21	63	7	21	63
Dose (ppm)									
Control	9	6	5	4163	3920	2816	18	16	12
35	8*	7	6	4200	4093	3193**	18	15	12
100	8	7	5	4099	3962	3314**	19	16	11
300	7**	7	6	4192	4029	3080	18	15	12
900	8*	7	6	4130	4210**	3335*	17	16	13

* = $p < 0.05$ as compared to control.
** = $p < 0.01$ as compared to control.

[a] = Data extracted from Bayer AG Report No. 11231, Tables 19, 20, 21 and Appendix.

Thyroid weight changes generally reflected the lower body weights seen for the treated animals, except that their weights on day 7 were relatively more affected than total body weights since the relative, as well as absolute organ weights were decreased. Liver weights, particularly for day 63, were notable in that they did not follow the pattern of body weight loss but were increased in all treated groups for both absolute and relative values, the latter being significantly increased for the 35, 100 and 900 ppm groups. Although absolute adrenal weights were significantly lower for the 900 ppm group on day 7 and for the 300 ppm on day 21, these changes reflected body weight changes and none of the relative adrenal weights was significantly different from controls for any interval.

2. Histological Examinations

According to the investigators: "No treatment-related pathological findings were obtained for the organs. . ." examined, however, there may have been changes in the "thyroid colloid". Table X below presents the findings for the thyroid. Individual animal data were provided by the investigators. These data show that staining of the thyroid colloid was altered in all treated groups and the incidence of this effect increased with duration of treatment.

Table X. Histological Observations - Thyroid Gland[a]
(10 animals per dose each time point)

Day:	7	21	63
Dose(ppm)			
Control	0	0	0
35	3k,3(k)	7k,3(k)	8k,2(k)
100	2k,3(k)	4k,5(k)	8k,2(k)
300	4k,3(k)	4k,6(k)	8k,2(k)
900	4k,0(k)	7k,2(k)	8k,2(k)

k = "Change in stainability of thyroid colloid; colloid mostly with loose and netlike structure."
(k) = "Transitional stage of changed stainability of thyroid colloid."
[a] = Data extracted from Bayer AG Report No. 11231, Appendix.

IV. CONCLUSIONS:

The treatment of male rats with DIC 1468 revealed systemic effects in all four dose groups, however, effects other than body weight and food consumption were generally not dose related. The systemic No Observed Effect Level (NOEL) for this study is below 35 ppm, the lowest dose tested.

The main thrust of this study was to establish the dose-time-effect relationship for DIC 1468 in the thyroid. From the data presented the investigators assumed ". . . that peripheral deiodination of thyroxine to triiodothyronine is impaired after administration of up to 900 ppm DIC 1468, so that a secondary increase in thyroid functioning takes place." However, the assumption is difficult to follow from the evidence presented. As stated by the investigators, the results did not present a pattern typical of hyperthyroid activity. Furthermore, usually no dose-response relationship for the effects was apparent although there was a 25 fold difference between the lowest and highest dose. However, it follows that treatment with DIC 1468 appears to have an effect on thyroid function, but the mechanism and biological significance remain unclear.

Perhaps the investigators should have measured the T/S ratio, the ratio of the iodide ion in the thyroid gland to that in the serum. This ratio is used to assess thyroid gland activity (iodine trapping). Also, a description of the configuration of the epithelial cells lining the thyroid follicles would have been helpful. These cells change their configuration depending on the state of activity of the thyroid gland.

V. CORE-CLASSIFICATION: Core Supplementary Data.

This study examined only males and the histopathology was limited in scope; furthermore, these data are ancillary and, as such, do not meet any regulatory requirement.

METRIBUZIN

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